

CLAIMS

What is claimed is:

- 5 1. An isolated and purified protein comprising an amino acid sequence as depicted in Figure 2 or 4 (SEQ ID NO: 2 or 4) and analogs thereof wherein the protein is capable of complexing with glial cell line-derived neurotrophic factor (GDNF) and thereby mediating cell response to GDNF.
- 10 2. A protein of Claim 1 comprising the amino acid sequence as depicted in Figure 2 (SEQ ID NO: 2).
3. A protein of Claim 1 comprising the amino acid sequence as depicted in Figure 4 (SEQ ID NO:4).
- 15 4. A protein of Claim 1 comprising the amino acid sequence Ser¹⁸ through Pro⁴⁴⁶ as depicted in Figure 2 (SEQ ID NO:2).
5. A protein of Claim 1 comprising the amino acid sequence Asp²⁵ through Leu⁴⁴⁷ as depicted in Figure 2 (SEQ ID NO:2).
- 20 6. A protein of Claim 1 comprising the amino acid sequence Cys²⁹ through Cys⁴⁴² as depicted in Figure 2 (SEQ ID NO:2).
7. A protein of Claim 1 comprising the amino acid sequence Ala¹⁹ through Val⁴⁵⁰ as depicted in Figure 4 (SEQ ID NO:4).
8. A protein of Claim 1 comprising the amino acid sequence Cys²⁹ through Cys⁴⁴³ as depicted in Figure 4 (SEQ ID NO:4).
- 30 9. A protein of Claim 1 which is glycosylated.
10. A protein of Claim 1 which is non-glycosylated.
- 35 11. A protein of Claims 1 to 10 which is produced by recombinant technology or chemical synthesis.

0886354.053097

Sub B'

5

10

15

depic
B

20

25

Sub
B2

30

35

19. A vector comprising a nucleic acid sequence encoding a neurotrophic factor receptor protein comprising the amino acid sequence as depicted in Figure 2 or 4

[illegible]

- Sub
B3 30

30. A method of claim 28, wherein said nucleic acid sequence encodes a neurotrophic factor receptor protein comprising the amino acid sequence as depicted in Figure 4 (SEQ ID NO:4).

(a) culturing a host cell transformed or transfected with a nucleic acid sequence according to claim 17 under conditions suitable for the expression of said neurotrophic factor receptor protein by said host cell; and

(b) optionally, isolating said neurotrophic factor receptor protein expressed by said host cell.

33. A method of claim 28 or 31, wherein said host cell is a prokaryotic cell.

35. A substantially purified neurotrophic factor receptor protein prepared according to the method of any of claims 28 to 31.

37. A method of treating improperly functioning dopaminergic nerve cells by administering a neurotrophic factor receptor protein of claim 1.

39. A method of treating Alzheimer's disease by administering a neurotrophic factor receptor protein of claim 1.

40. A method of treating amyotrophic lateral sclerosis by administering a

41. An antibody that binds to a neurotrophic factor receptor protein comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID NO:4.

42. The antibody of claim 41 wherein said antibody is a monoclonal antibody.

43. The antibody of claim 41 wherein said antibody is a polyclonal antibody.

10 44. An antibody produced by immunizing an animal with a neurotrophic factor
receptor protein comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID
NO:4.

45. A hybridoma that produces a monoclonal antibody that binds to a neurotrophic
15 factor receptor protein comprising an amino acid sequence of SEQ ID NO:2 or SEQ ID
NO:4.

46. A device for treating nerve damage, comprising:

(a) a semipermeable membrane suitable for implantation; and

20 (b) cells encapsulated within said membrane, wherein said cells secrete a neurotrophic factor receptor protein according to claim 1;

said membrane being permeable to the neurotrophic factor receptor protein and impermeable to materials detrimental to said cells.

25 47. The device of claim 46, wherein said cells are naturally occurring cells that secrete said neurotrophic factor receptor protein.

48. The device of claim 46, wherein said cells have been modified to secrete said neurotrophic factor receptor protein by means of a nucleic acid sequence comprising:

30 (a) a sequence set forth in Figure 1 (SEQ ID NO.: 1) comprising nucleotides encoding Met¹ through Ser⁴⁶⁵ or Figure 3 (SEQ ID NO: 3) comprising nucleotides encoding Met¹ through Ser⁴⁶⁸ encoding a neurotrophic factor receptor protein (GDNFR) capable of complexing with glial cell line-derived neurotrophic factor (GDNF) and mediating cell response to GDNF;

35 (b) a nucleic acid sequence which (1) hybridizes to a complementary sequence of (a) and (2) encodes an amino acid sequence with GDNFR activity; and

(c) a nucleic acid sequence which but for the degeneracy of the genetic code would

hybridize to a complementary sequence of (a) and (2) encodes an amino acid sequence with GDNFR activity.

49. An assay device for analyzing a test sample for the presence of glial cell line-derived neurotrophic factor, comprising: a solid phase containing or coated with a GDNFR protein, wherein said GDNFR protein reacts with GDNF present in the test sample and produces a detectable reaction product indicative of the presence of GDNF.

50. A method for analyzing a test sample for the presence of glial cell line-derived neurotrophic factor, comprising: contacting the sample to an assay reagent comprising GDNFR protein, wherein said GDNFR protein reacts with GDNF present in the test sample and produces a detectable reaction product indicative of the presence of GDNF.

51. An isolated and purified protein comprising an amino acid sequence of GDNFR- α , GRR2, GRR3 or GDNFR consensus protein as depicted in Figure 14, 15, 16, 17, 18, 19 or 26 wherein the protein is capable of complexing with glial cell line-derived neurotrophic factor (GDNF) or neurturin neurotrophic factor thereby mediating cell response to said neurotrophic factor.

52. A pharmaceutical composition comprising a protein as claimed in claim 51 in combination with a pharmaceutically acceptable carrier.

53. An isolated nucleic acid sequence encoding a neurotrophic factor receptor protein comprising an amino acid sequence claim 51.

54. An isolated nucleic acid sequence comprising:

- (a) a sequence set forth in Figure 19 or 26 wherein said sequence encodes a neurotrophic factor receptor protein (GDNFR) capable of complexing with glial cell line-derived neurotrophic factor (GDNF) or neurturin neurotrophic factor thereby mediating cell response to said neurotrophic factor;
- (b) a nucleic acid sequence which (1) hybridizes to a complementary sequence of (a) and (2) encodes an amino acid sequence with GDNFR activity; and
- (c) a nucleic acid sequence which but for the degeneracy of the genetic code would hybridize to a complementary sequence of (a) and (2) encodes an amino acid sequence with GDNFR activity.

55. A vector comprising a nucleic acid sequence according claims 53 or 54

operatively linked to one or more operational elements capable of effecting the amplification or expression of said nucleic acid sequence.

56. A host cell transformed or transfected with the vector of claim 55.

57. A host cell of Claim 56 wherein said cell is suitable for human implantation and wherein said cell expresses and secretes said neurotrophic factor receptor.

58. A host cell of Claim 56 wherein said cell is transformed or transfected ex vivo.

59. A host cell of Claim 56 wherein said cell is enclosed in a semipermeable membrane suitable for human implantation.

60. A method for the production of a neurotrophic factor receptor protein comprising the steps of:

- (a) culturing a host cell, containing a nucleic acid sequence encoding a neurotrophic factor receptor protein comprising an amino acid sequence of claim 51 wherein the protein is capable of complexing with glial cell line-derived neurotrophic factor (GDNF) or neurturin neurotrophic factor thereby mediating cell response to said neurotrophic factor, under conditions suitable for the expression of said neurotrophic factor receptor protein by said host cell; and
- (b) optionally, isolating said neurotrophic factor receptor protein expressed by said host cell.

61. A method of treating improperly functioning dopaminergic nerve cells by administering a neurotrophic factor receptor protein of claim 51.

62. An antibody that binds to a neurotrophic factor receptor protein comprising an amino acid sequence of claim 51.

63. A hybridoma that produces a monoclonal antibody that binds to a neurotrophic factor receptor protein comprising an amino acid sequence of claim 51.

64. A device for treating nerve damage, comprising:

- (a) a semipermeable membrane suitable for implantation; and
- (b) cells encapsulated within said membrane, wherein said cells secrete a

neurotrophic factor receptor protein according to claim 51;
said membrane being permeable to the neurotrophic factor receptor protein and impermeable to materials detrimental to said cells.

5 65. An assay device for analyzing a test sample for the presence of a neurotrophic factor, comprising: a solid phase containing or coated with a GDNFR protein, wherein said GDNFR protein reacts with said neurotrophic factor present in the test sample and produces a detectable reaction product indicative of the presence of neurotrophic factor.

10

66. A method for analyzing a test sample for the presence of a neurotrophic factor, comprising: contacting the sample to an assay reagent comprising GDNFR protein, wherein said GDNFR protein reacts with said neurotrophic factor present in the test sample and produces a detectable reaction product indicative of the presence of

15

67. A method of determining whether a ligand activates a receptor tyrosine kinase, comprising: contacting the sample to an assay reagent comprising GDNFR protein, wherein said GDNFR protein reacts with said ligand to form a GDNFR protein/ligand complex and wherein said complex binds to an extracellular ligand-binding domain of said receptor tyrosine kinase, and detecting the activation of the kinase domain and phosphorylation of specific substrates that mediate intracellular signaling.

20

68. A method of claim 67, wherein said receptor tyrosine kinase is a c-ret proto-oncogene.

25

69. A method of claim 67, wherein a cell has been modified to include the extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic segment containing the catalytic protein-tyrosine kinase domain for the detection of intracellular signaling.

30

08866354 053097

Add B⁴Add C¹